In The Name of God

STEM CELL

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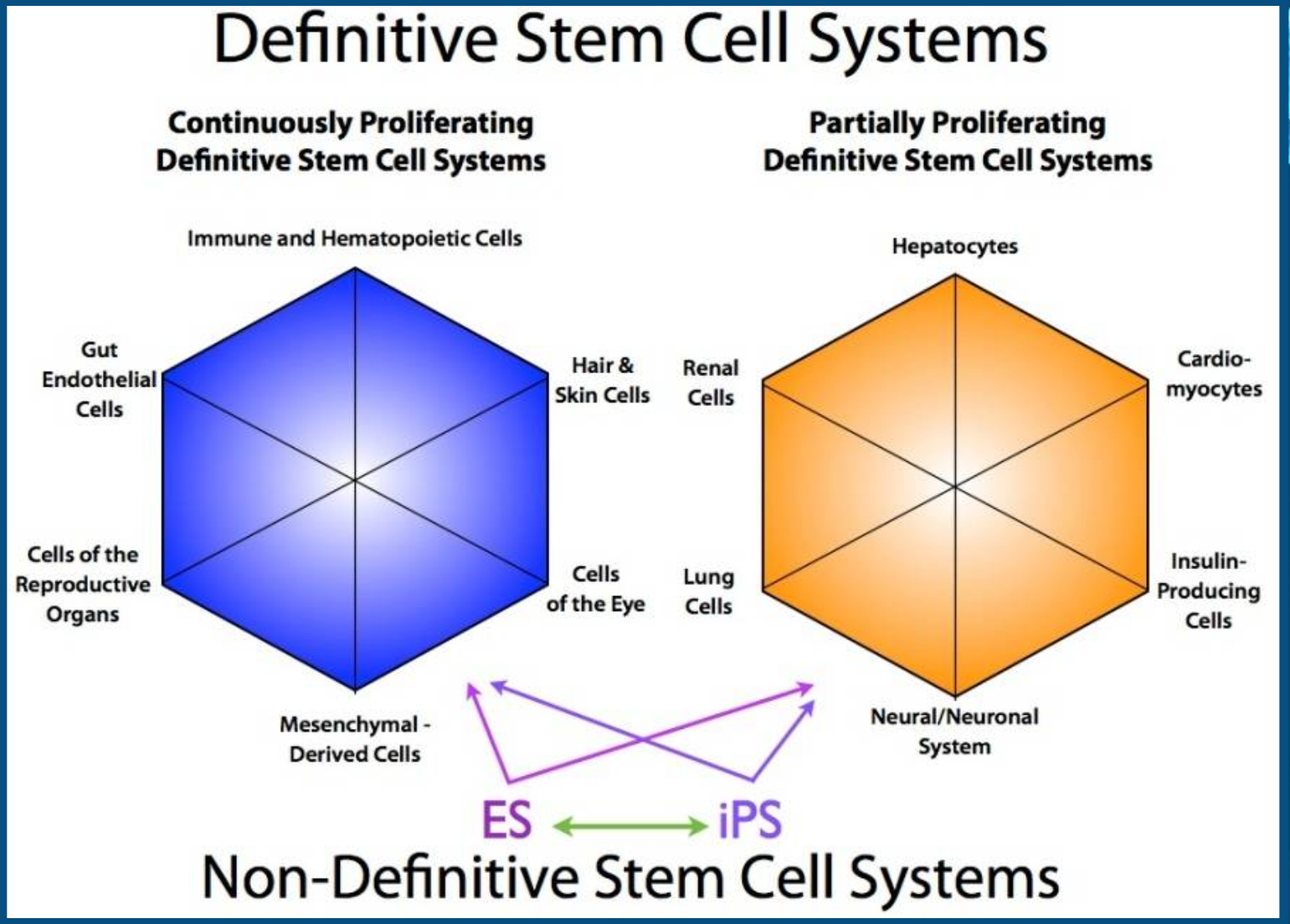
Content

What Are Stem Cells? [1]

- An undifferentiated cell of a multicellular organism.
- Remarkable potential to develop into different cell types.
- Capable of giving rise to more cells of the same type.
- Other kinds of cell arise by differentiation.
- Capacity to self-renew.
- **Potency (Toti / Pluri / Multi / Unipotent)***
- Clonality, functional parameters.

What Are Stem Cells?

- Internal repair system, dividing essentially without limit.
- Two types, namely non-definitive and definitive stem cells.
- Non-definitive, capacity of developing into any organ. [2]
- The best example for non-definitive: fertilized egg.
- Most commonly known: embryonic stem cells (ESC) and induced pluripotent stem (iPS) cells.
- Definitive are derived from non-definitive, tissue specific.



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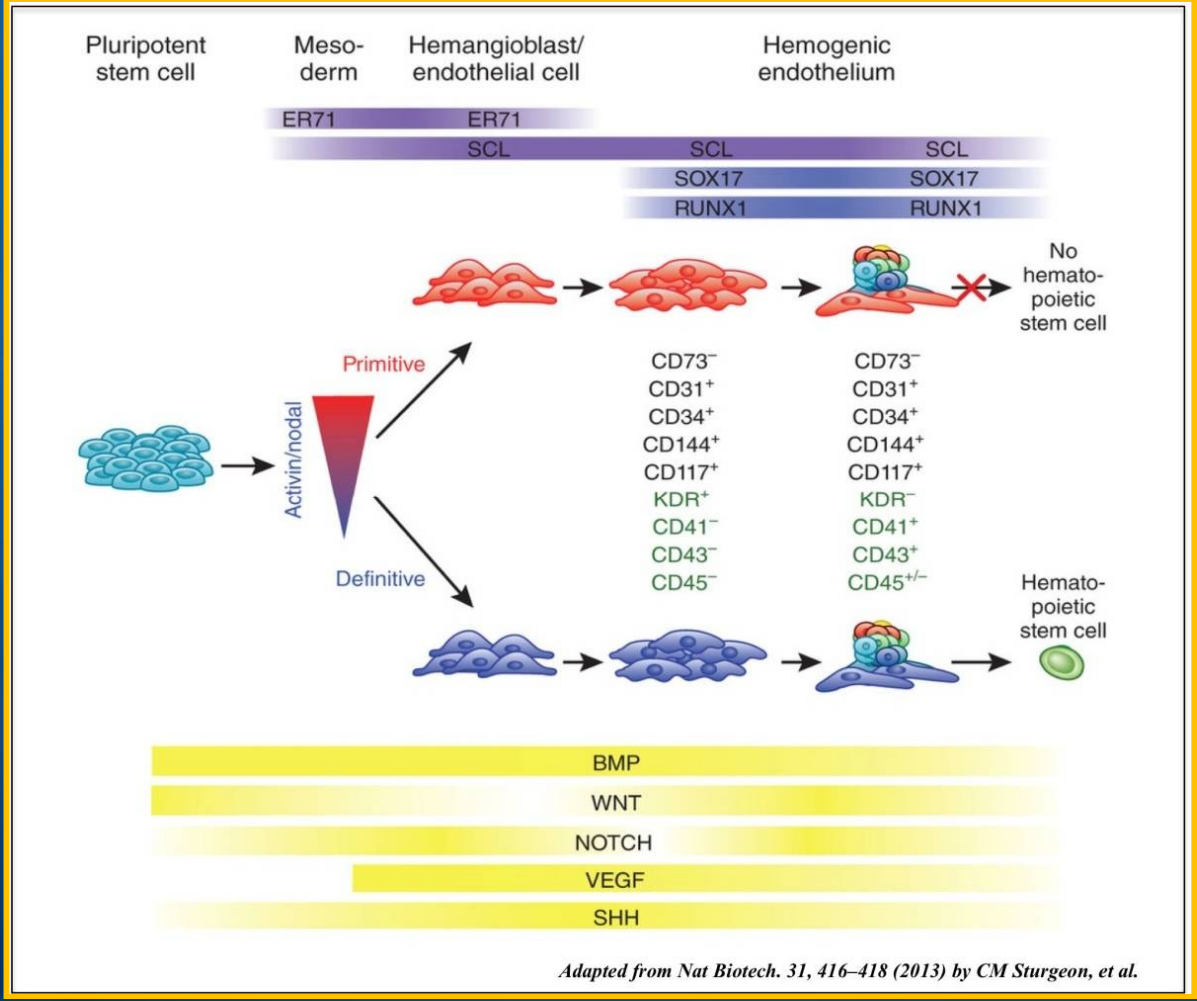
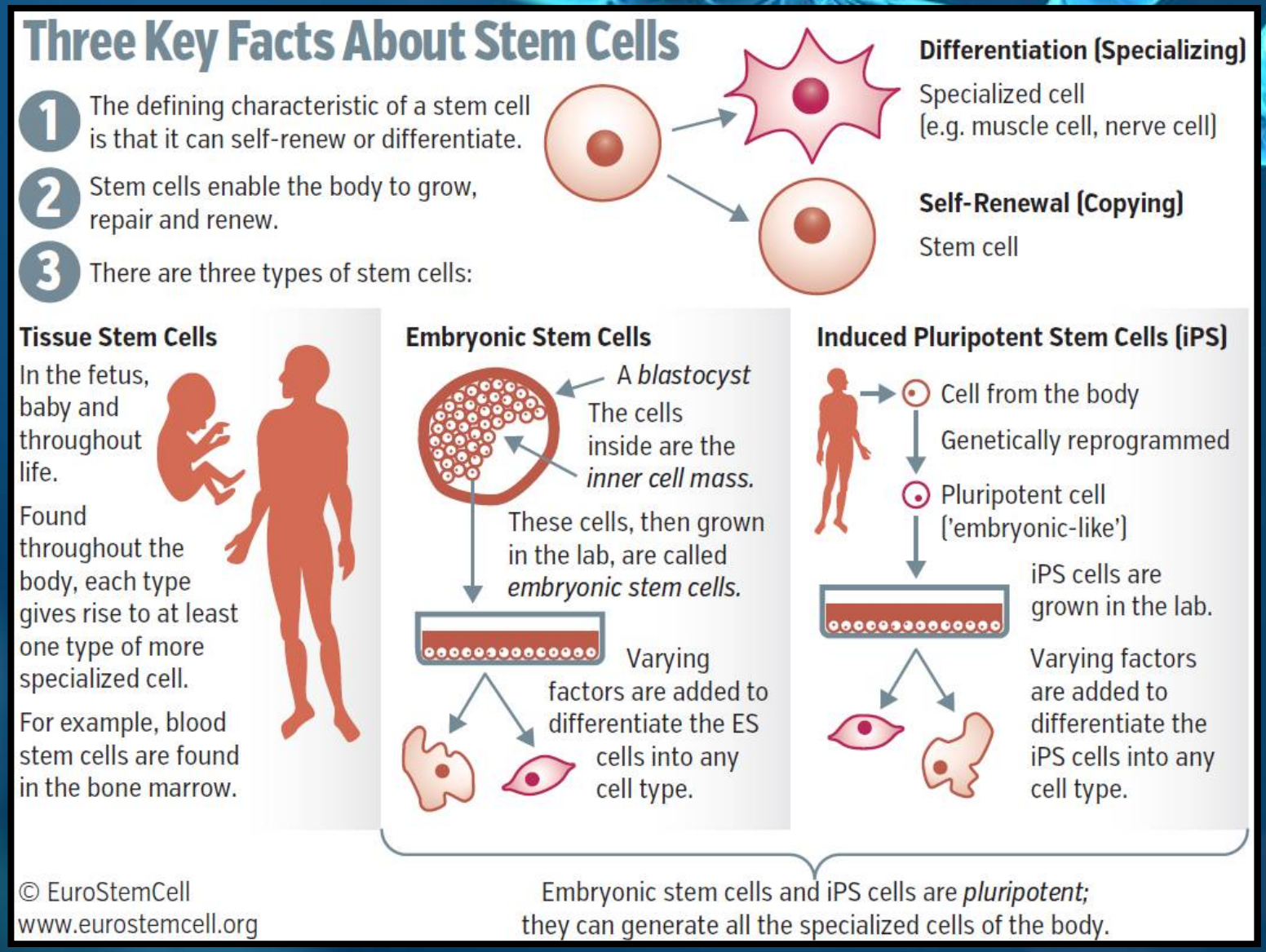


http://www.intechopen.com/books/pluripotent-stem-cell-biology-advances-in-mechanisms-methods-and-models/human-embryonic-stem-cell-derived-primitive-and-definitive-hematopoiesis

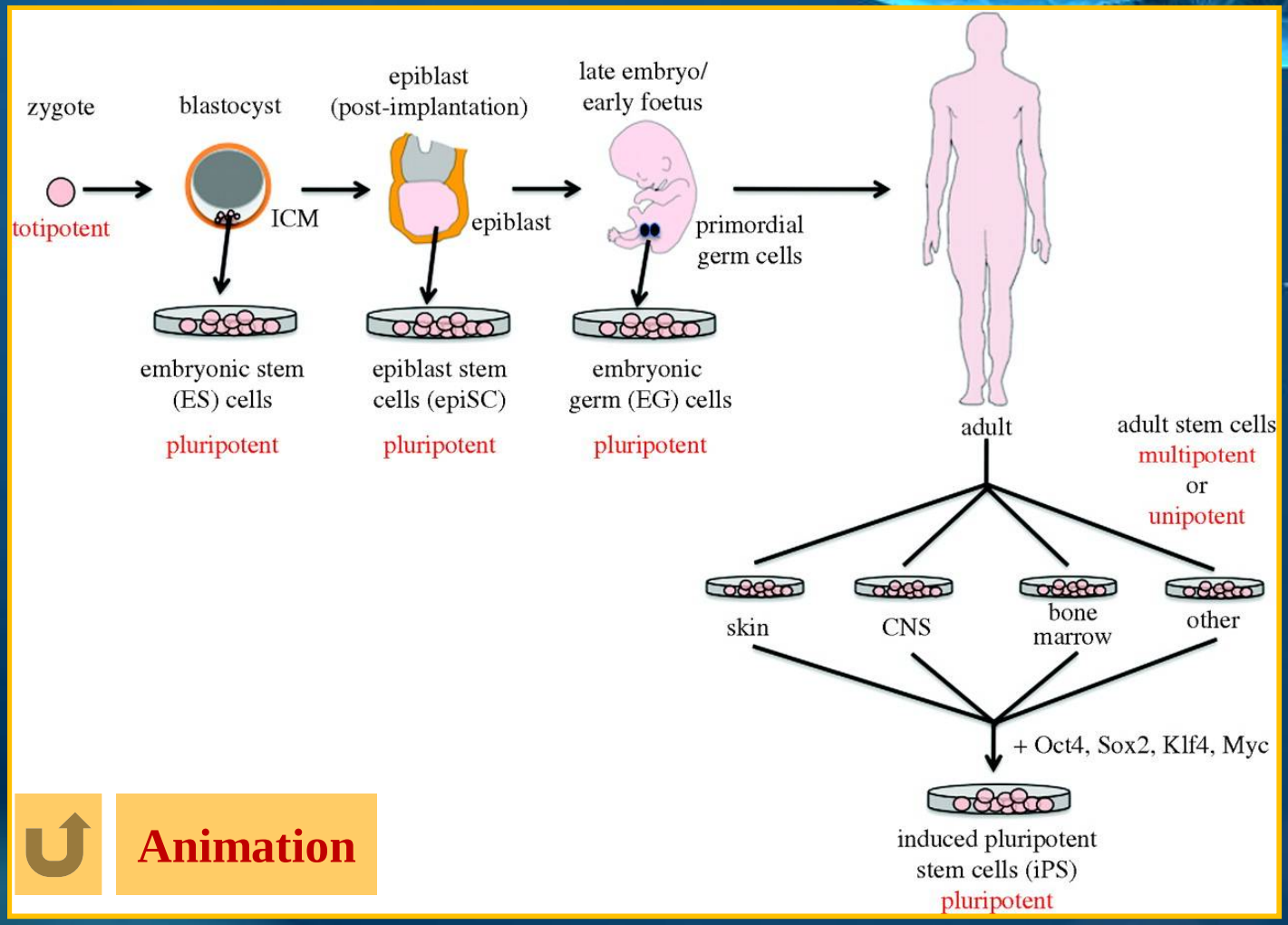


http://www.researchtrends.com/issue-36-march-2014/stem-cell-research/

Potency [1,7]

- **Totipotent** (zygote, morula (*cdx2*, *oct3/4*), any cell, whole body, three layers, placenta (*cdx2*))
- **Pluripotent** (toti-derrived, ESCs, not whole body, three layers, multipotent cells)
- **Multipotent** (pluri-derrived, ASCs (HSCs and MSCs))
- **Unipotent or Committed** (one type, self-renewal, tissue-specific stem cells)

Img & Animation



http://rstb.royalsocietypublishing.org/content/365/1537/155

Why Stem Cell Research?[1]

- The origin of stem cell technology
- Applications of stem cells in medicine
- Challenges to the use of stem cells

Why Stem Cell Research?[1]

- **The origin of stem cell technology**
  - John Gurdon, Ian Wilmut , *reprogramming* amphibian cells.
  - Martin Evans, *pluripotent* embryonic stem cells.
  - Irv Weismann, adult *hematopoietic* stem cells, bone marrow transplants.
  - Arthur Caplan, *mesenchymal* SCs, *multipotent* capacities.
  - Regenerative medicine.

Why Stem Cell Research?[1]

- **The origin of stem cell technology**
- **Applications of stem cells in medicine**
  - Induced pluripotent stem (iPS), new insights, mental retardation, autism, epilepsy, and schizophrenia.
  - Biotechnology industry.
  - Adult cell-based therapies, ameliorating genetic diseases.

Why Stem Cell Research?[1]

- The origin of stem cell technology
- Applications of stem cells in medicine
- **Challenges to the use of stem cells**
  - Vocal and manipulative conservative and religious interest groups.
  - Rigorous design, If not, the hypotheses will fail.

Stem Cells Origins

- The origin or lineage of stem cells: embryonic stem (ES) cells.
- 1998, by Thomson's group, the number of different hESC lines registered [2]
- More than 800 different hESC lines are now currently available [11]
- Origin of adult stem cells is less clear and controversial.

Types of Stem Cells

- 1. Adult Stem Cells (ASC) or Tissue-specific Stem Cells
- 2. Fetal Stem Cells
- 3. Cord Blood Stem Cells
- 4. Embryonic Stem Cells (ESC)
- 5. Induced Pluripotent Stem Cells (iPS cells) [3]

Types of Stem Cells [9]

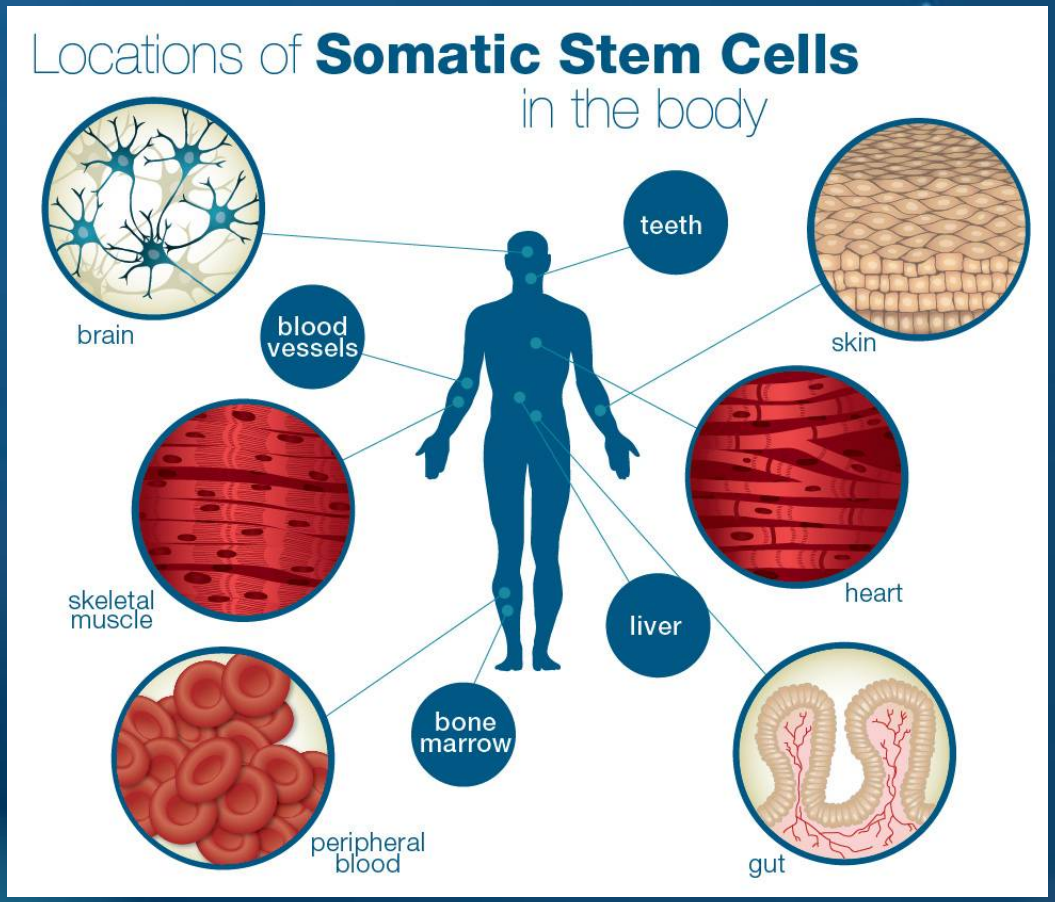
Name	Sources	Advantages	Disadvantages
Neural Stem Cells	Brain and spinal cord	1. Multipotent: can differentiate into neurons, astrocytes, and oligodendrocytes	1. Limited differentiation potential
		2. Show tumor-tropic properties for various cancers	2. Limited source
Hematopoietic Stem Cells	Bone marrow, cord blood, peripheral blood	1. Multipotent: can form lymphoid and myeloid blood cells	Limited differentiation potential
		2. Many sources	
		3. Most well-established stem cell source	
Mesenchymal Stem Cells	Bone marrow, adipose tissue, cord blood	1. Multipotent – readily differentiates into bone, cartilage, fat, and muscle but can also be induced to differentiate into neuronal cells	1. Limited differentiation potential but better than NSCs and HSCs
		2. Many sources	2. Immunosuppressive properties
Embryonic Stem Cells	Inner cell mass of blastocyst	Pluripotent – has the highest differentiation potential	1. Ethically controversial source (destruction of embryos)
			2. Teratoma formation in vivo (requires ex vivo differentiation prior to transplantation)
Induced Pluripotent Stem Cells	Somatic cells	1. Pluripotent: has similar differentiation potential as ESCs	1. Potential tumorigenicity
		2. Can be derived from many cell types	2. Low reprogramming efficiency
		3. Patient-specific	3. Characteristics are protocol dependent

Yin, P. T., et al. (2015). "Engineering Stem Cells for Biomedical Applications." Advanced healthcare materials.

Adult Stem Cells or Tissue-specific Stem Cells

- Many adult tissues contain stem cells.
- Replace cells that die or restore tissue after injury.
- Adult stem cells are tissue-specific.
- The term ‘adult stem cells’ is used very broadly.
- May include fetal and cord blood stem cells.
- Few stem cell therapies are widely accepted. [3]

Adult Stem Cells or Tissue-specific Stem Cells



http://learn.genetics.utah.edu/content/stemcells/quickref/

Fetal Stem Cells

- Are taken from the fetus.
- From approximately 10 weeks of gestation.
- Most tissues in a fetus contain stem cells.
- Drive the rapid growth and development of the organs.
- Like adult stem cells, tissue-specific. [3]

Embryonic Stem Cells

- Are derived from very early embryos, after a blastocyst, [1]
- Can in theory give rise to all cell types.
- Carry the risk of transforming into cancerous tissue after transplantation.
- No treatments using embryonic stem cells are accepted. [3]

Induced Pluripotent Stem Cells (iPS cells)

- Are created by inducing the specialized cells (like skin).
- Express genes that are normally made in ES cells.
- ES cells and iPS cells share many characteristics.
- ES and iPS cells are not identical; sometimes behave slightly differently.
- Powerful method for creating patient- and disease-specific cell lines for research.

In vivo Reprogramming! [10]

- ASC to iPS opened new therapeutic opportunities
- Little is known about the possibility of *in vivo* reprogramming
- Induction of ***Oct4***, ***Sox2***, ***Klf4*** and ***c-Myc*** in mice, teratomas
- Full reprogramming can occur *in vivo**
- Analyses of the stomach, intestine, pancreas and kidney:

  - Expression of the pluripotency marker NANOG, indicative of *in situ* reprogramming

In vivo Reprogramming! [10]

Generation of **reprogrammable** mice

Reprogrammable mice generate **teratomas**

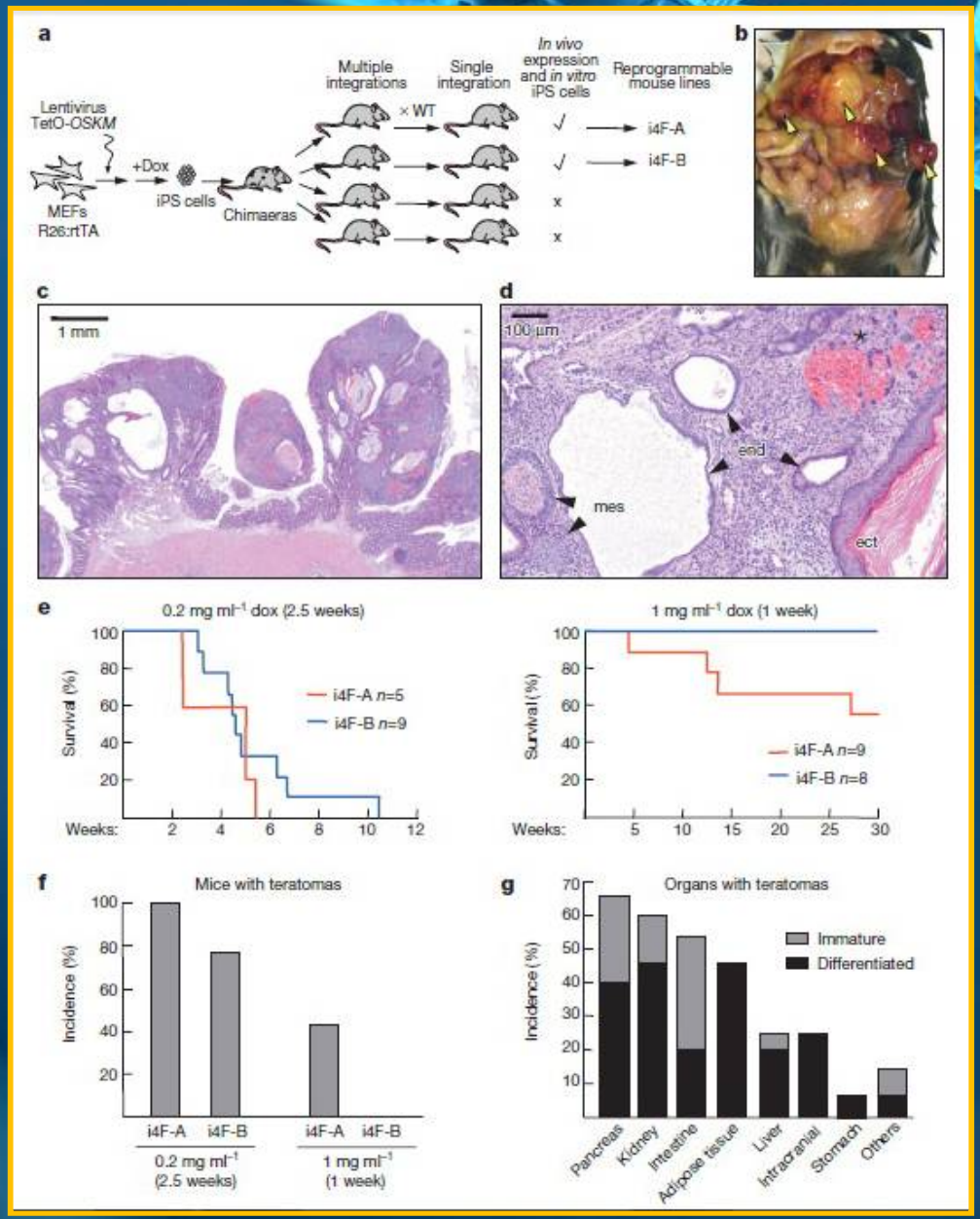
In vivo reprogramming occurs in **multiple tissues**

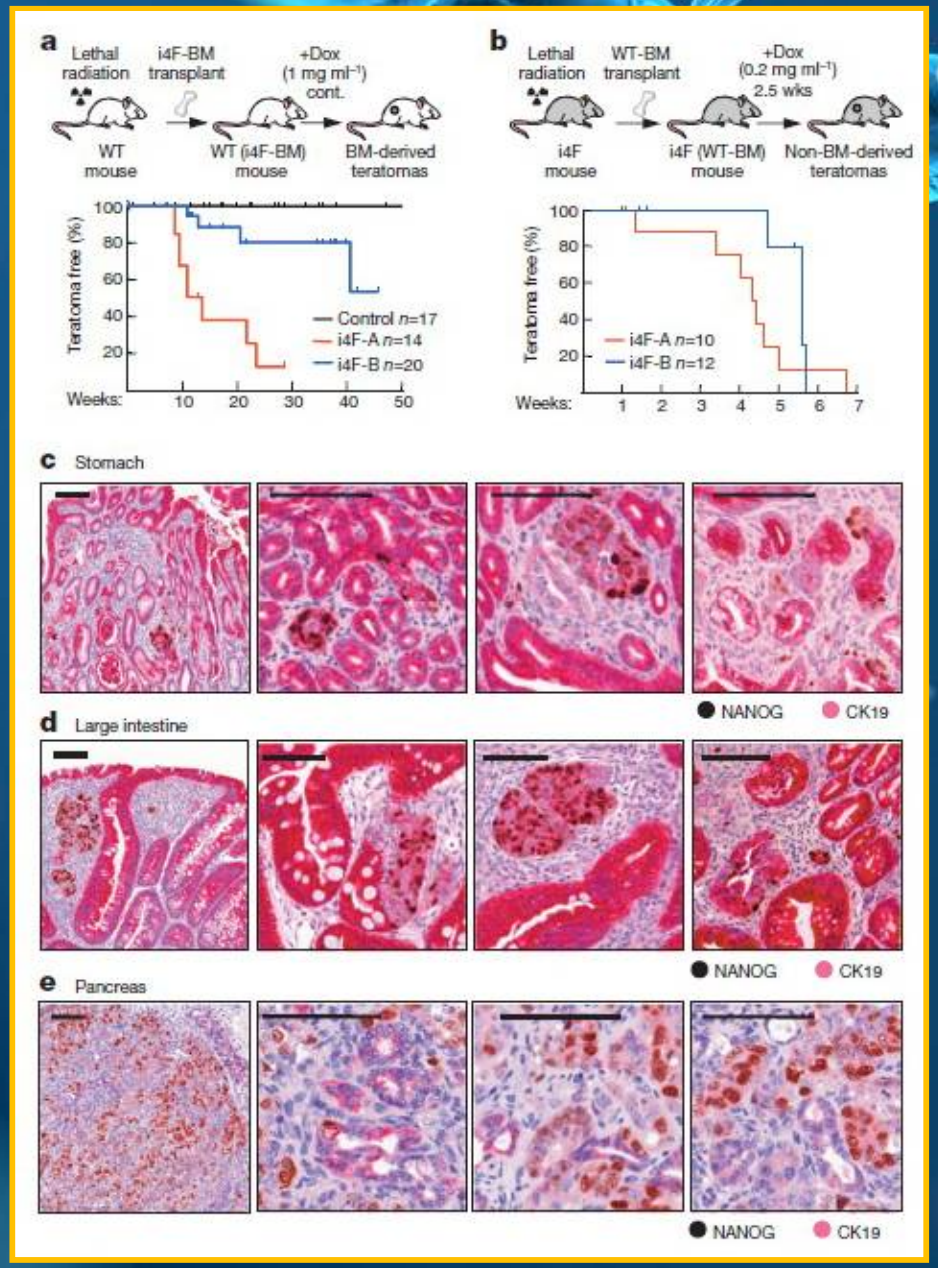
Reprogrammable mice present **iPS** cells in the **blood**

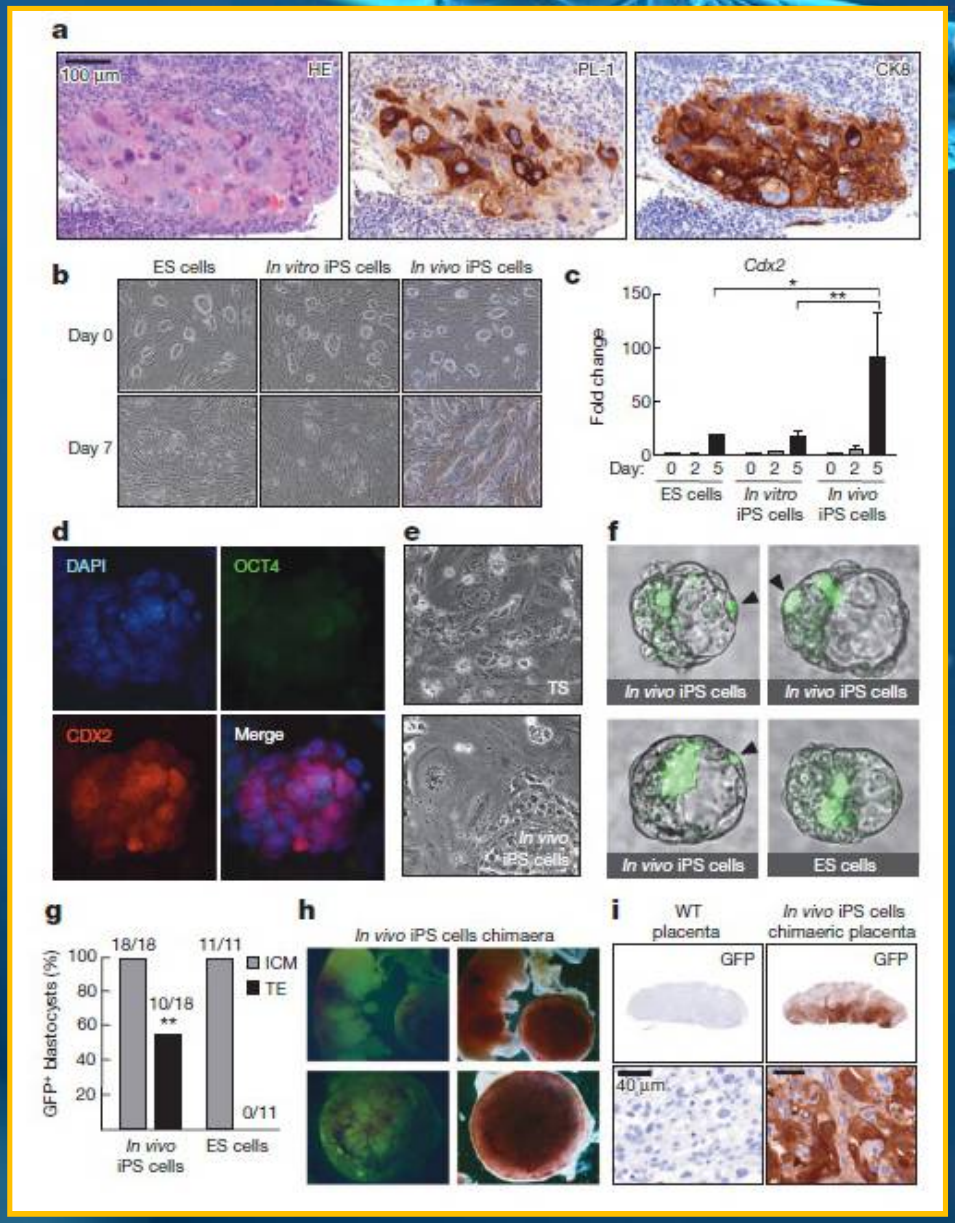
Transcriptomic analysis of *in vivo* iPS cells

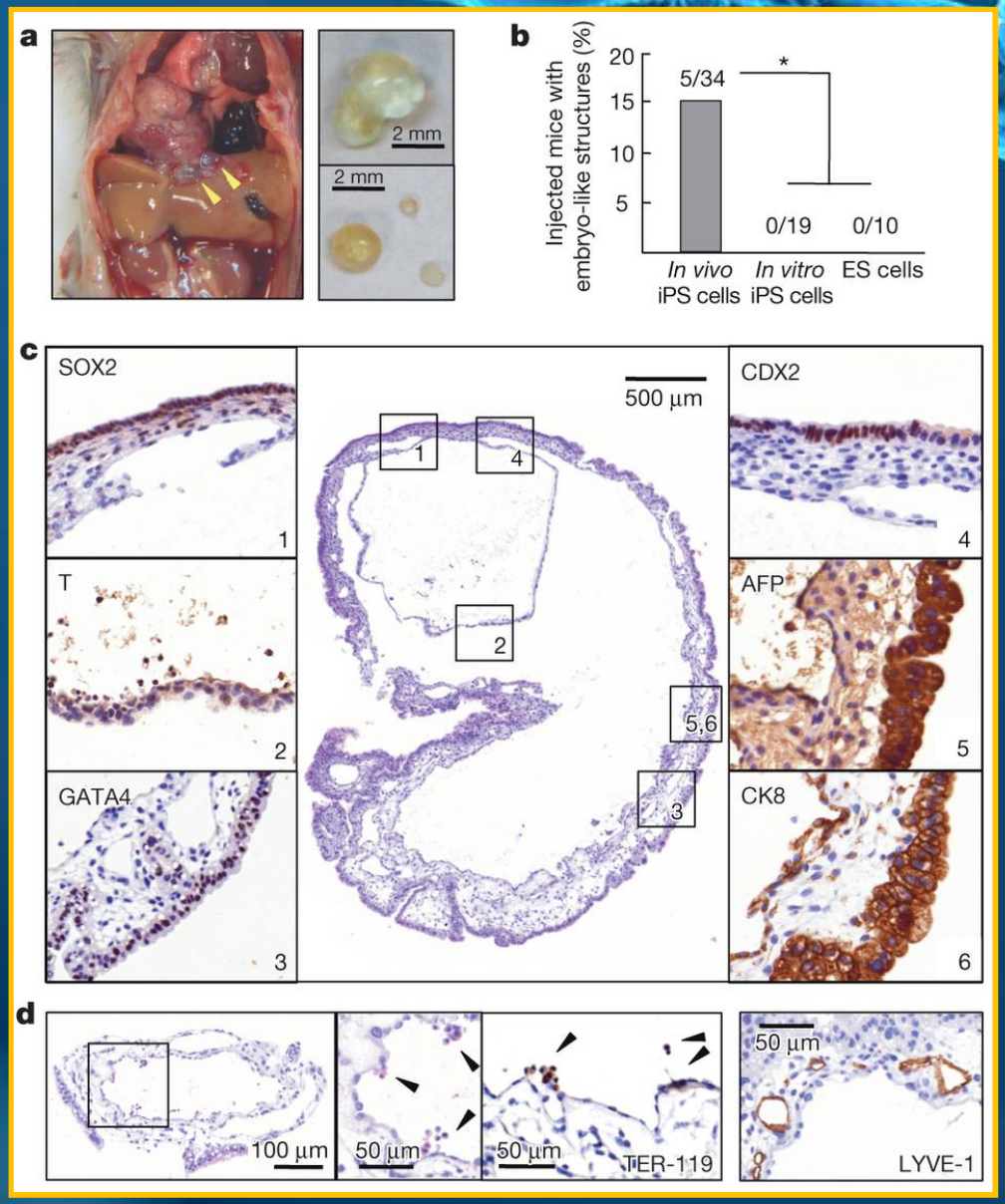
In vivo iPS cells contribute to the **trophectoderm**

In vivo iPS cells generate **embryo-like** structures


a
Lentivirus
TetO-OSKM
MEFs
R26:rtTA
+Dox
iPS cells
Chimaeras
Multiple integrations
Single integration
× WT
In vivo expression and in vitro iPS cells
Reprogrammable mouse lines
√
i4F-A
√
i4F-B
x
x
b
c
1 mm
d
100 μm
end
mes
ect
e
0.2 mg ml⁻¹ dox (2.5 weeks)
Survival (%)
i4F-A n=5
i4F-B n=9
Weeks:
1 mg ml⁻¹ dox (1 week)
Survival (%)
i4F-A n=9
i4F-B n=8
Weeks:
f
Mice with teratomas
Incidence (%)
i4F-A
i4F-B
i4F-A
i4F-B
0.2 mg ml⁻¹ (2.5 weeks)
1 mg ml⁻¹ (1 week)
g
Organs with teratomas
Incidence (%)
Immature
Differentiated
Pancreas
Kidney
Intestine
Adipose tissue
Liver
Intracranial
Stomach
Others


a
Lethal radiation
i4F-BM transplant
+Dox (1 mg ml⁻¹) cont.
WT mouse
WT (i4F-BM) mouse
BM-derived teratomas
Teratoma free (%)
Weeks:
Control n=17
i4F-A n=14
i4F-B n=20
b
Lethal radiation
WT-BM transplant
+Dox (0.2 mg ml⁻¹) 2.5 wks
i4F mouse
i4F (WT-BM) mouse
Non-BM-derived teratomas
i4F-A n=10
i4F-B n=12
c Stomach
d Large intestine
e Pancreas
NANOG
CK19


a
100 µm
HE
PL-1
CK8
b
ES cells
In vitro iPS cells
In vivo iPS cells
Day 0
Day 7
c
Cdx2
Fold change
150
100
50
0
Day:
0 2 5 0 2 5 0 2 5
ES cells
In vitro iPS cells
In vivo iPS cells
d
DAPI
OCT4
CDX2
Merge
e
TS
In vivo iPS cells
f
In vivo iPS cells
In vivo iPS cells
In vivo iPS cells
ES cells
g
GFP+ blastocysts (%)
100
80
60
40
20
18/18
10/18
**
11/11
0/11
ICM
TE
In vivo iPS cells
ES cells
h
In vivo iPS cells chimaera
i
WT placenta
In vivo iPS cells chimaeric placenta
GFP
GFP
40 µm


a
2 mm
2 mm
b
Injected mice with embryo-like structures (%)
20
15
10
5
5/34
*
0/19
0/10
In vivo iPS cells
In vitro iPS cells
ES cells
c
SOX2
1
T
2
GATA4
3
500 µm
1
4
2
5,6
3
CDX2
4
AFP
5
CK8
6
d
100 µm
50 µm
50 µm
TER-119
50 µm
LYVE-1

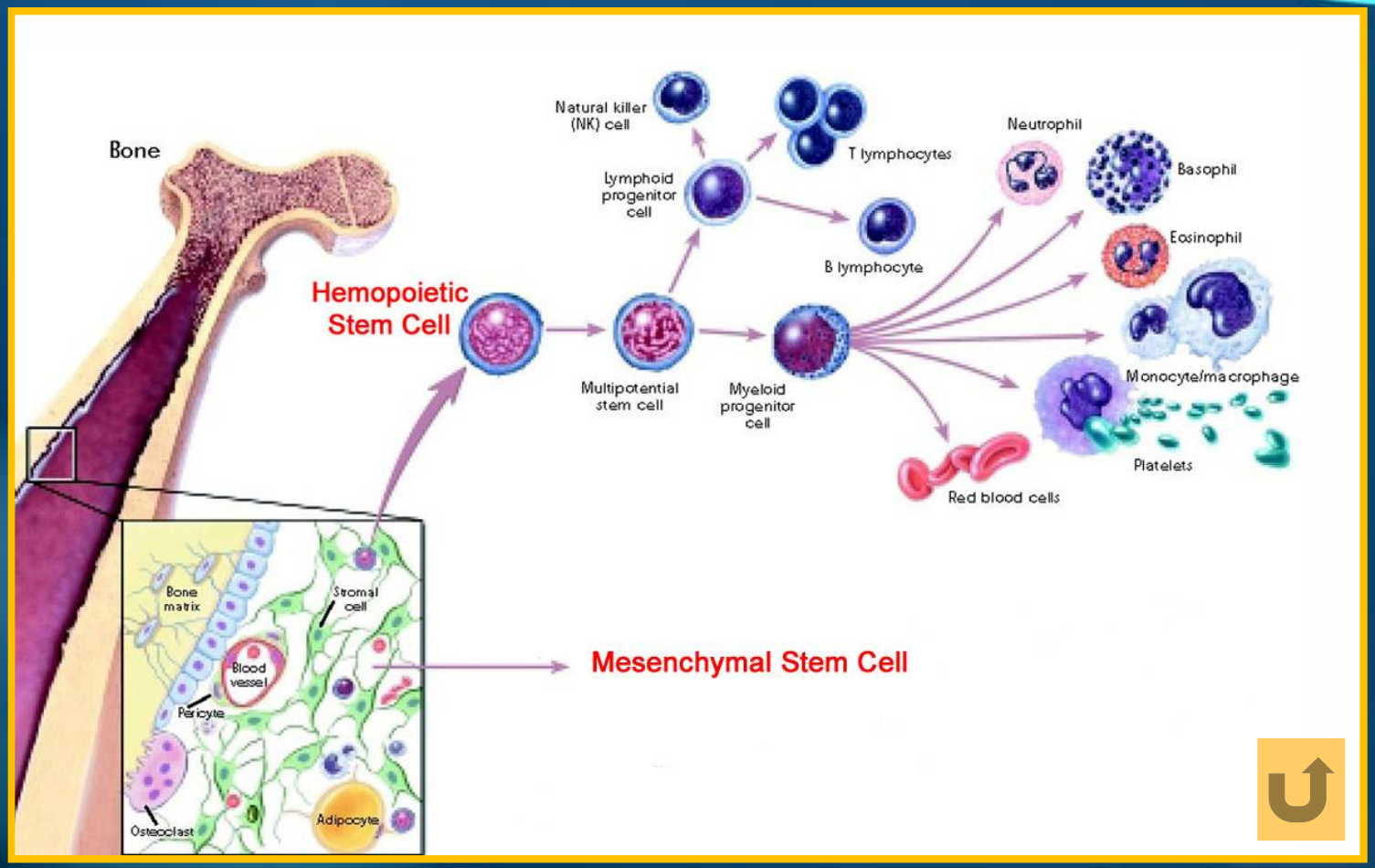
Stem Cells Sources [7]

- Bone Marrow (BM) **HSCs and MSCs**
- Peripheral Blood (PB) – $CD34^{+}/38^{-}/lin^{-}$/Thy-1

  - **Apheresis** method (advantages / disadvantages)

- Embryo (blastocyst or aborted)
- **Menstrual Blood** [12]
- Umbilical Cord Blood (UCB)

Bone Marrow



http://biblescripture.net/stemcell.html

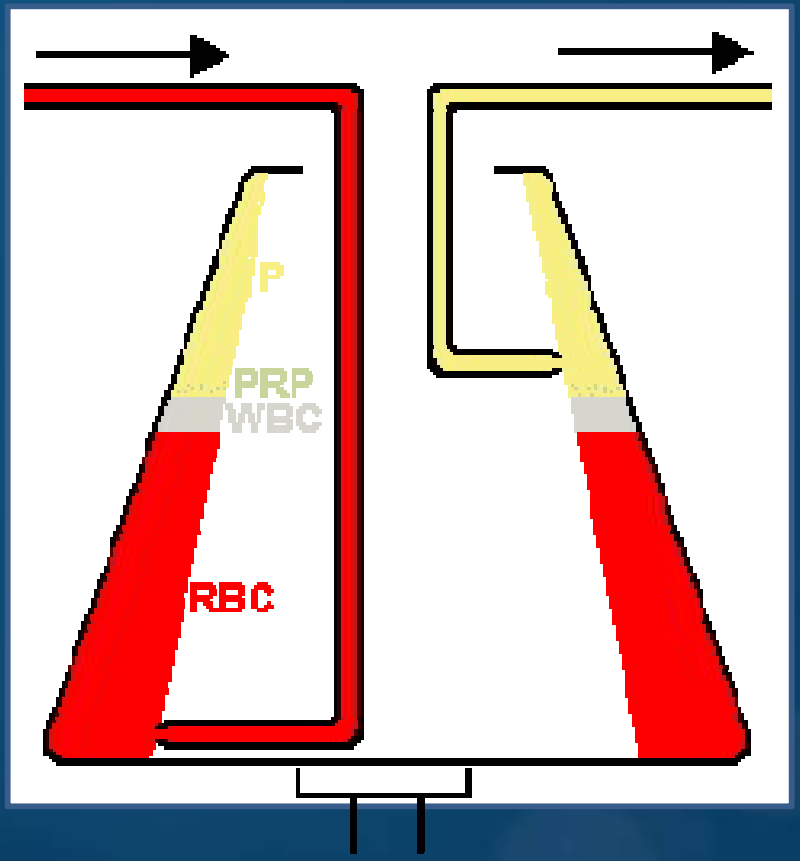
Peripheral Blood (PB)

Apheresis Method: [13]

- Removal of whole blood from a patient or donor
- The components of whole blood are separated
  - Plasma (plasmapheresis)
  - Platelets (plateletpheresis)
  - Leukocytes (leukapheresis)

Apheresis [13]

1) Whole blood is introduced into a chamber that is spinning
2) Blood separates into components (P = plasma; PRP = platelet rich plasma; WBC = leukocytes; RBC = red blood cells)
3) By gravity along the wall of the chamber. The component to be removed can be selected by moving the level of the aspiration device at the right.



Apheresis

- **Advantages:**

  - Easy to perform, no anesthesia, multiple blood volume processing

- **Disadvantages:**

  - Long time to have stem cells, central vein for high volume, infections, etc

Menstrual Blood [12]

- Easily accessible and inexpensive source of stem cells (MenSCs).
- Substitution of fetal bovine serum (FBS) with human platelet derivatives (HPDs)
- Mineralization was significantly higher in cells differentiated in the presence of HPR

Cord Blood (CB) Stem Cells

- The blood in the umbilical cord.
- Rich in blood-forming stem cells.
- The applications are similar to those of adult bone marrow.
- Tissue-specific.
- CB can be collected without risk to the mother or infant donor.
- Can be cryopreserved for several decades for future use. [4]

Cord Blood (CB) Stem Cells

- Before delivery of the placenta (in utero) or after it (ex utero).
- Distinct advantages over bone marrow transplantation (BMT)
- Use of UCB cells in transplantation depends on the number of stem cells.

Advantages over BMT and Disadvantages

Some Advantages: [5]

- Safety of procurement with minimal risk to the donor.
- A more lenient HLA-matching requirement.
- A lower incidence of graft-versus-host disease.

Disadvantages: [4]

- Presence of delayed engraftment kinetics.
- A higher rate of engraftment failure than BMT or Peripheral Blood Transplantation (PBT).

UCB Stem Cells vs. Adult Stem Cells [6]

- UCB-derived hematopoietic cells:

  Higher proliferation and expansion potentials.

  Capacity to self-renew is also superior .

- Differences in telomere dynamics and cell cycle progression:

  UCB cells possess longer telomeres

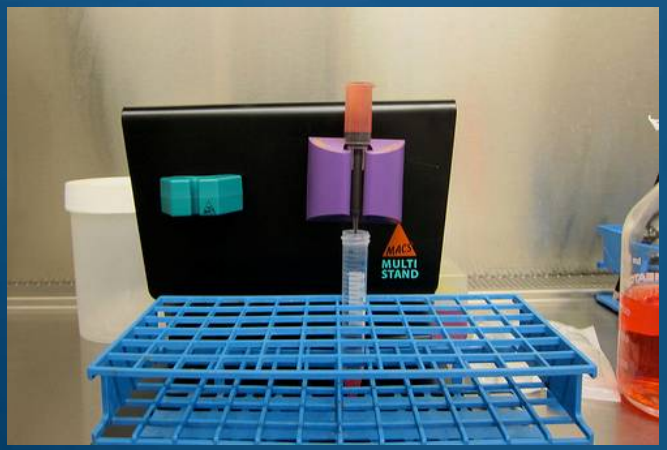
UCB Stem Cells vs. Adult Stem Cells [6]

- Express higher levels of certain cell cycle regulators.
- Level of certain transcription factor pathways,
- Differential gene expression profiles
- the autocrine production of particular cytokines.

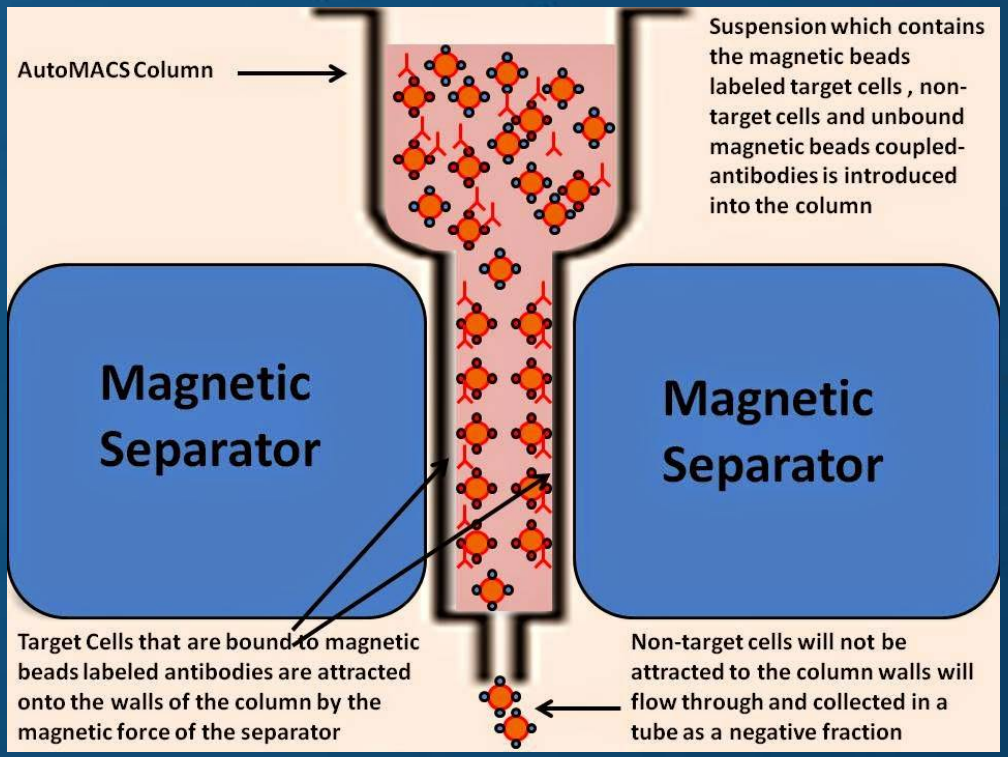
MACS Column [7]

- Magnetic Activated Cell Sorting
- UCB stem cell separation
- Soft matrix, cell friendly, micro beads, labeling
- Columns: XS, LS, MS, D, CS, DS

MACS Column [7]



http://findingmykd.blogspot.com/2013/02/macs-column.html



A Short Clip

http://textbookhaematology4medical-scientist.blogspot.com/2014/06/pbmc-isolation-using-miltenyi-biotec.html

Stem Cell
Ethical Considerations [14]

- Source of stem-cells.
- Newborn placental or umbilical cord blood or adult bone-marrows without loss of natural life is ethical.
- Creation of embryos by in-vitro fertilization
- Killing a human being for the survival of another.

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THANKS FOR YOUR ATTENTION
stem cells